

Amendments to the Claims:

Please amend claim 34 to remove the comma from "preferentially, aminoacylating in the last section of the claim.

Please amend claim 41 to include an "and" between Markush members b) and c).

These amendments introduce no new matter and support for the amendment is replete throughout the specification and claims as originally filed. The amendments are merely administrative to correct sentence structure in the claims. These amendments are made without prejudice and are not to be construed as abandonment of the previously claimed subject matter, or agreement with any objection or rejection of record.

This listing of claims will replace all prior versions, and listings, of claims in the application.

Listing of Claims:

Claims 1 to 33 (Cancelled).

34. (Currently amended) A method of incorporating a 5-substituted tryptophan (HTTP) or 5-substituted tryptophan unnatural amino acid into a peptide, the method comprising:

preparing a construct comprising a nucleic acid sequence encoding an orthogonal tRNA synthetase (O-RS) and comprising at least 90% identity to the sequence of SEQ ID NO: 2, the O-RS comprising a proline residue at a position corresponding to position 144 of SEQ ID NO: 2, wherein the O-RS aminoacylates a reference orthogonal tRNA (O-tRNA) of SEQ ID NO: 3 with a 5-HTP or a 5-substituted tryptophan analog when the reference O-tRNA, 5-HTP or 5-substituted tryptophan analog, and the O-RS are present in a eukaryotic cell;

preparing a construct comprising a nucleic acid sequence encoding an O-tRNA comprising: at least 90% identity to SEQ ID NO: 3, wherein the O-tRNA is aminoacylated with the 5-HTP or 5-substituted tryptophan analog by a reference O-RS of SEQ ID NO: 2 when the reference RS, 5-HTP or 5-substituted tryptophan analog, and the O-tRNA are present in eukaryotic cell;

introducing the O-RS construct and the O-tRNA construct into the eukaryotic cell; and,

preferentially[[,]] aminoacylating an expressed O-tRNA with the unnatural amino acid, wherein said aminoacylation is catalyzed by an expressed O-RS, wherein the O-tRNA recognizes a selector codon in a nucleic acid sequence encoding the peptide,

whereby the 5-HTP or 5-substituted tryptophan unnatural amino acid is incorporated into the peptide in the eukaryotic cell.

35. (Previously presented) The method of claim 34, wherein the unnatural amino acid is 5-hydroxy-L-tryptophan (5-HTPP).

36. (Previously presented) The method of claim 35, further comprising applying a voltage to the peptide, thereby reacting the 5-HTPP with a reactive molecule in the peptide.

37. (Original) The method of claim 36, wherein reacting comprises cross-linking.

38. (Original) The method of claim 36, wherein the reactive molecule comprises an unnatural amino acid in another peptide.

39. (Original) The method of claim 34, further comprising detecting an interaction between the peptide and another peptide.

40. (Original) The method of claim 39, wherein said detecting comprises fluoroscopy.

41. (Currently amended) The method of claim 34, wherein the O-RS construct comprises a nucleic acid comprising a polynucleotide sequence selected from the group consisting of:

a) a coding polynucleotide sequence of SEQ ID NO: 1,

b) a coding polynucleotide sequence that encodes a polypeptide of SEQ ID NO: 2,
and

c) a complementary sequence of (a) or (b).

42. (Previously presented) The method of claim 34, wherein the O-RS construct comprises a mutated tryptophanyl-tRNA synthetase peptide sequence mutated at one or more amino acid residues based on structure data of the tryptophanyl-tRNA synthetase or an analogous aminoacyl-tRNA synthetase.

43. (Original) The method of claim 42, wherein the mutated tryptophanyl-tRNA synthetase comprises a *Bacillus* tryptophanyl-tRNA synthetase mutated at Val144.

44. (Previously presented) The method of claim 34, wherein the O-tRNA construct comprises a polynucleotide sequence selected from the group consisting of: SEQ ID NO: 3, and a complementary polynucleotide sequence thereof.

45. (Original) The method of claim 34, wherein said preparing the O-tRNA construct comprises inclusion of one or more tRNA flanking sequences that functionally interact with an RNA polymerase of the cell.

46. (Original) The method of claim 34, wherein the O-tRNA construct comprises an A box eukaryotic transcriptional control element.

47. (Previously presented) The method of claim 34, further comprising mutating the O-tRNA sequence to include a functional A box eukaryotic transcriptional control element.

48. (Original) The method of claim 47, wherein said mutating comprises site directed mutagenesis.

49. (Previously presented) The method of claim 34, wherein the O-tRNA construct or O-RS construct comprises: a reporter tag or a purification tag.

50. (Previously presented) The method of claim 34, wherein the O-RS encoding construct and the O-tRNA encoding construct are comprised in the same construct.

51. (Cancelled)

52. (Original) The method of claim 34, further comprising transfecting a nucleic acid encoding the peptide into the cell.

53. (Previously presented) The method of claim 52, wherein the cell comprises a mammalian cell.

54. (Previously presented) The method of claim 34, further comprising expressing the O-RS construct or the O-tRNA construct.

55. (Previously presented) The method of claim 54, further comprising purifying expressed O-RS or expressed O-tRNA.

Claims 56 to 62 (Cancelled).

63. (Previously presented) The method of claim 34, wherein the O-RS comprises at least 95% identity to SEQ ID NO: 2.

64. (Previously presented) The method of claim 34, wherein the O-RS comprises at least 98% identity to SEQ ID NO: 2.

65. (Previously presented) The method of claim 34, further comprising mutating and screening a nucleic acid encoding the amino acid sequence of SEQ ID NO: 2 to obtain the O-RS.

66. (Previously presented) The method of claim 34, wherein the O-RS comprises with two adjacent binding pockets separated by an a-helix peptide consisting of Asp at a position corresponding to position 140, Ile at a position corresponding to position 141, Val at a position corresponding to position 142, Pro at a position corresponding to position 143, Gly at a position corresponding to position 145.

67. (Previously presented) The method of claim 34, wherein the O-RS comprises Ser at a position corresponding to position 7, His at a position corresponding to position 44, and Asp at a position corresponding to position 133.

68. (Previously presented) The method of claim 46, wherein the A box eukaryotic transcriptional control element comprises: G at a position corresponding to position 7, G at a position corresponding to position 9, or U at a position corresponding to position 11.

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69. (Previously presented) The method of claim 34, wherein the O-tRNA comprises at least 95% identity to the sequence of SEQ ID NO: 3.